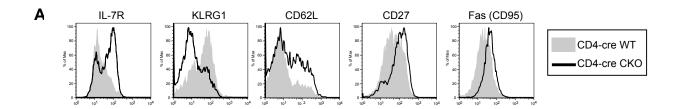


Figure S1. Deletion efficiency in Blimp-1 CKO mice.

To assess the frequency of LCMV-specific CD8 T cells that have deleted *Prdm1*, GzB-cre⁺; *Prdm1*^{flox/flox} mice were crossed to ROSA26:EYFP transgenic mice in which expression of enhanced yellow fluorescent protein (EYFP) gene is blocked by an upstream loxP-flanked STOP sequence. In these mice, deletion of the STOP sequence and thus transcription of EYFP is initiated in cells that express the Cre recombinase under the control of the human Granzyme B promoter. Mice were infected with LCMV and LCMV-specific CD8 T cells were analyzed for EYFP fluorescence by flow cytometry 5, 7, or 81 days p.i. in the peripheral blood. Histogram plots gated on D^bGP₃₃₋₄₁+ CD8 T cells (as shown in left plot) show that 85-95% of virus-specific CD8 T cells were EYFP+ and thus underwent Cre-mediated deletion at this locus. A population of CD8-CD44- cells is shown for comparison in grey histograms. Of note, of all cells in the spleen that activate the EYFP reporter at day 7 post-infection, 85-90% are CD8+, ~5% are CD4+ (~10-20% of total CD4+ population), and ~2% are NK1.1+.



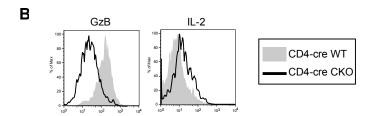


Figure S2. Phenotype of Blimp-1 deficient P14 CD8 T cells in CD4-cre CKO system. 50,000 Ly5.2+ CD4-cre⁺; *Prdm1*^{flox/flox} (CD4-cre CKO) or CD4-cre⁺; *Prdm1*^{+/+} (CD4-cre WT) P14 cells were transferred i.v. into Ly5.1+ recipient mice that were subsequently infected with LCMV. Ly5.2+ LCMV-specific effector CD8 T cells were evaluated 8 days later for expression of IL-7R, KLRG1, CD62L, CD27, Fas (CD95) (A) Granzyme B (GzB), and IL-2 (B) by flow cytometry. IL-2 was analyzed by ICCS after 5 hour *in vitro* stimulation with GP₃₃₋₄₁ peptide. Similar results were obtained when Ick-cre⁺; *Prdm1*^{flox/flox} P14 cells were used. These animals had no detectable virus in the serum at day 5 after infection.

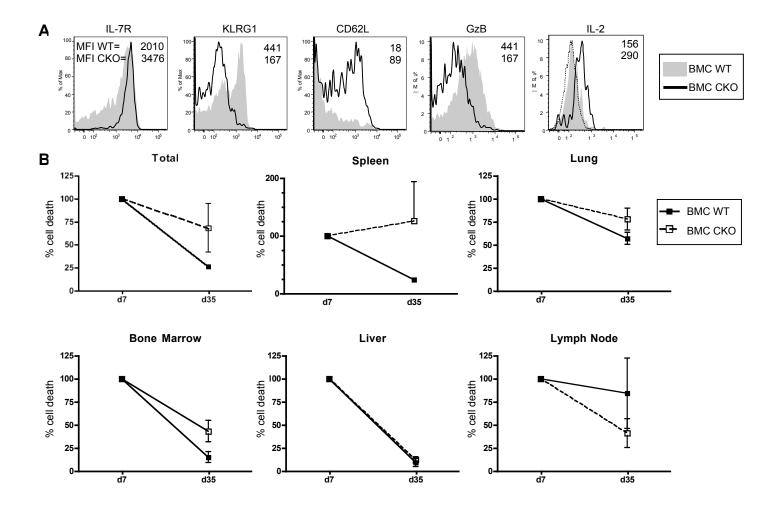


Figure S3. Phenotype and contraction of Blimp-1 deficient CD8 T cells in mixed bone marrow chimeras.

To better isolate the Blimp-1 deficiency to CD8 T cells, bone marrow chimeras (BMC) were created by mixing bone marrow (BM) in a 90:10 ratio from CD8 α -/- animals and either Ly5.2+ GzB-cre⁻; $Prdm1^{flox/flox}$ (BMC WT) or Ly5.2+ GzB-cre⁺; $Prdm1^{flox/flox}$ (BMC CKO) mice and transferring the BM into congenic (Ly5.1+) mice. After 8 weeks, mice were infected with LCMV and the phenotype and numbers of LCMV-specific CD8 T cells were analyzed at days 7 and 35 p.i.

- (A) The phenotype of Ly5.2+CD8+D^bGP₃₃₋₄₁+ cells from BMC at day 7 after LCMV infection was determined by staining splenocytes with antibodies to IL-7R, KLRG1, CD62L, Granzyme B and IL-2 (after 5hr ICCS; unstimulated control, dotted line) and analyzing cells via FACS (mean MFI is shown). Similar results were obtained in other tissues and also for D^bNP₃₉₆₋₄₀₄-specific CD8 T cells. There was no detectable virus in the serum or spleens of these animals at this time point (data not shown).
- (B) The relative rate of LCMV-specific effector CD8 T cell death during the contraction phase, between days 7 and 35 post LCMV infection, was compared between WT and CKO CD8 T cells. To normalize across the animals due to differences in % chimerism, the average combined number of Ly5.2+ D^bGP₃₃₋₄₁+ and D^bNP₃₉₆₋₄₀₄+ CD8 T cells present at day 7 p.i. was set at 100%, and then the average number of cells remaining at day 35 is shown as a percent of the day 7 numbers. Note: Although the fold contraction was greater in the lymph nodes of the BMC CKO animals, BMC CKO animals did maintain higher overall numbers of cells in the lymph nodes at both d7 and d35 compared to WT animals. This experiment was performed once with 2-3 animals in each group per time point.

Table S1: Complete list of genes significantly differentially expressed between WT and Blimp-1 CKO effector CD8 T cells

Genes more highly expressed in CKO vs WT cells

d change KO/WT	Gene Symbol	Gene Name	Accessio Number
13.4	Ccr7	Mus musculus chemokine (C-C motif) receptor 7 (Ccr7)	NM_007719.
11.7	Nsg2	Mus musculus neuron specific gene family member 2 (Nsg2)	NM_008741.
9.6	Tacstd2	Mus musculus tumor-associated calcium signal transducer 2 (Tacstd2)	NM_020047.
9.2	Prdm1	Mus musculus PR domain containing 1, with ZNF domain (Prdm1)	NM_007548.
8.5	Xcl1	Mus musculus chemokine (C motif) ligand 1 (XcI1)	NM_008510.
6.9	ldb3	Mus musculus inhibitor of DNA binding 3 (Idb3)	NM_008321.
6.4	Tcf7	Mus musculus transcription factor 7, T-cell specific (Tcf7)	NM_009331.
5.7	Ccr6	Mus musculus chemokine (C-C motif) receptor 6 (Ccr6)	NM_009835.
5.6	4930583H14Rik	Mus musculus RIKEN cDNA 4930583H14 gene (4930583H14Rik)	NM_026358.
4.4	Cxcl10	Mus musculus chemokine (C-X-C motif) ligand 10 (Cxcl10)	NM_021274
4.0	Enpp2	Mus musculus ectonucleotide pyrophosphatase/phosphodiesterase 2 (Enpp2)	NM_015744
3.5	Dkk3	Mus musculus dickkopf homolog 3 (Xenopus laevis) (Dkk3)	NM 015814.
3.4	Rgs10	Mus musculus regulator of G-protein signalling 10 (Rgs10)	NM 026418.
3.3	C330003B14Rik	Mus musculus RIKEN cDNA C330003B14 gene (C330003B14Rik)	NM 175342
3.3	Adk	Mus musculus adenosine kinase (Adk)	NM 134079.
3.2	Tnfrsf26	Mus musculus tumor necrosis factor receptor superfamily, member 26 (Tnfrsf26)	NM 175649.
3.2	Tnfrsf4	Mus musculus tumor necrosis factor receptor superfamily, member 2 (Trifrsf4)	NM 011659
3.1	Kit	Mus musculus kit oncogene (Kit)	NM 021099
3.0	Gucy1a3		NM_021896.
		Mus musculus guanylate cyclase 1, soluble, alpha 3 (Gucy1a3)	
2.9	Usp18	Mus musculus ubiquitin specific protease 18 (Usp18)	NM_011909.
2.9	Pacsin1	Mus musculus protein kinase C and casein kinase substrate in neurons 1 (Pacsin1)	NM_011861.
2.9	Cables1	Mus musculus Cdk5 and Abl enzyme substrate 1 (Cables1)	NM_022021.
2.9	Tnfsf4	Mus musculus tumor necrosis factor (ligand) superfamily, member 4 (Tnfsf4)	NM_009452
2.9	Gstt3	Mus musculus glutathione S-transferase, theta 3 (Gstt3)	NM_133994.
2.9	Lrig1	Mus musculus leucine-rich repeats and immunoglobulin-like domains 1 (Lrig1)	NM_008377.
2.8	Serpina3g	Mus musculus serine (or cysteine) proteinase inhibitor, clade A, member 3G (Serpina3g)	XM_354694.
2.8	Crtam	Mus musculus cytotoxic and regulatory T cell molecule (Crtam)	NM_019465.
2.8	C330008K14Rik		XM_128979
2.8	II7r	Mus musculus interleukin 7 receptor (II7r)	NM_008372
2.8	F2rl1	Mus musculus coagulation factor II (thrombin) receptor-like 1 (F2rl1)	NM_007974
2.6	Egr2	Mus musculus early growth response 2 (Egr2)	NM 010118.
2.6	C330008K14Rik		XM 128979.
2.6	Ifi205	Mus musculus interferon activated gene 205 (Ifi205)	NM 172648.
2.6	Armcx2	3-1-2-(NM 026139.
2.5	Tpi1		NM_009415.
2.5	2410129E14Rik	Mus musculus RIKEN cDNA 2410129E14 gene (2410129E14Rik)	NM_023716.
2.5	Slc2a6	Mus musculus solute carrier family 2 (facilitated glucose transporter), member 6 (Slc2a6)	NM_172659.
			NM 178595
2.4	2210013M04Rik	Mus musculus RIKEN cDNA 2210013M04 gene (2210013M04Rik)	
2.4	Cd9	Mus musculus CD9 antigen (Cd9)	NM_007657.
2.4	Tyki	Mus musculus thymidylate kinase family LPS-inducible member (Tyki)	NM_020557.
2.4	Adk	Mus musculus adenosine kinase (Adk)	NM_134079
2.4	Mif	Mus musculus macrophage migration inhibitory factor (Mif)	XM_147409.
2.4	Pou6f1	Mus musculus POU domain, class 6, transcription factor 1 (Pou6f1)	NM_010127.
2.4	2310061N23Rik	Mus musculus RIKEN cDNA 2310061N23 gene (2310061N23Rik)	NM_029803.
2.4	Sell	Mus musculus selectin, lymphocyte (Sell)	NM_011346.
2.4	Ddef1	Mus musculus development and differentiation enhancing (Ddef1)	NM_010026.
2.4	Fxyd4	Mus musculus FXYD domain-containing ion transport regulator 4 (Fxyd4)	NM_033648.
2.3	AA408556	Mus musculus expressed sequence AA408556 (AA408556)	NM_199447.
2.3	Gzmm	Mus musculus granzyme M (lymphocyte met-ase 1) (Gzmm)	NM_008504.
2.3	Slamf6		NM_030710.
2.3	Pscd3	Mus musculus pleckstrin homology, Sec7 and coiled-coil domains 3 (Pscd3)	NM 011182.
2.3	2310032F03Rik	Mus musculus RIKEN cDNA 2310032F03 gene (2310032F03Rik)	XM 130301.
2.3	Apex1	Mus musculus apurinic/apyrimidinic endonuclease 1 (Apex1)	NM 009687
2.3	Eva1		NM 007962
2.3	Ctla4	Mus musculus cytotoxic T-lymphocyte-associated protein 4 (Ctla4)	NM_009843.
2.3	Galk1	Mus musculus galactokinase 1 (Galk1)	NM_016905.
2.3	Mrps6	Mus musculus mitochondrial ribosomal protein S6 (Mrps6)	NM_080456.
		Inida mascalas mitocrionanal noosomal protein 36 (MfpS6)	
2.2	Igh-6	Mus museulus DIKEN aDNA 4700040D02 rana (4700040D02D2)	XM_354710.
2.2	1700019D03Rik	Mus musculus RIKEN cDNA 1700019D03 gene (1700019D03Rik)	NM_144953. NM 030682.
2.2	Tir1	Mus musculus toll-like receptor 1 (Tir1)	
2.2	Aicda	Mus musculus activation-induced cytidine deaminase (Aicda)	NM_009645.
2.2	Nme4	Mus musculus expressed in non-metastatic cells 4, protein (Nme4)	NM_019731.
2.2	lmmp2l	Mus musculus inner mitochondrial membrane peptidase 2-like (S cerevisiae) (Immp2l)	NM_053122
2.2	Tm4sf5	Mus musculus transmembrane 4 superfamily member 5 (Tm4sf5)	NM_029360
2.2	Tm4sf8	Mus musculus transmembrane 4 superfamily member 8 (Tm4sf8)	NM_019793
2.2	Bckdhb	Mus musculus branched chain ketoacid dehydrogenase E1, beta polypeptide (Bckdhb)	NM_199195.
2.1	5730469M10Rik	Mus musculus RIKEN cDNA 5730469M10 gene (5730469M10Rik)	NM_027464
2.1	lgh-6		XM_354710.
2.1	D730019B10Rik		NM_172785.
2.1	Dscr2	Mus musculus Down syndrome critical region homolog 2 (human) (Dscr2)	NM_019537.
2.1	6530401D17Rik	Mus musculus RIKEN cDNA 6530401D17 gene (6530401D17Rik)	NM_029823
2.1	Bcl6	Mus musculus B-cell leukemia/lymphoma 6 (Bcl6)	NM_009744.
2.1	Emb	Mus musculus embigin (Emb)	NM_010330.
2.1	Рур	Mus musculus pyrophosphatase (Pyp)	NM_026438.
2.1	1300017K07Rik	Mus musculus RIKEN cDNA 1300017K07 gene (1300017K07Rik)	NM_027919.
2.1		Mouse mRNA fragment for T-cell receptor beta chain variable region (cell line C5)	X01641.1
2.1	2410008K03Rik	Mus musculus RIKEN cDNA 2410008K03 gene (2410008K03Rik)	XM_125970
4.1	Car12		NM_178396
2.1		Mus musculus carbonic anyhydrase 12 (Car12)	
2.1	Tgn	Mus musculus thyroglobulin (Tgn)	NM_009375
2.1		Mus musculus phospholipid transfer protein (Pltp)	NM_011125
2.1	Pitp		NM_033042
2.1 2.0 2.0	Tnfrsf25	Mus musculus tumor necrosis factor receptor superfamily, member 25 (Tnfrsf25)	
2.1 2.0 2.0 2.0	Tnfrsf25 Ndg2		NM_175329.
2.1 2.0 2.0 2.0 2.0	Tnfrsf25 Ndg2 Cd83	Mus musculus CD83 antigen (Cd83)	NM_175329. NM_009856.
2.1 2.0 2.0 2.0	Tnfrsf25 Ndg2		NM_175329. NM_009856.
2.1 2.0 2.0 2.0 2.0	Tnfrsf25 Ndg2 Cd83	Mus musculus CD83 antigen (Cd83)	NM_175329. NM_009856. NM_013694. NM_023418. NM_025840.

Genes more highly expressed in WT vs CKO cells

Fold change WT/CKO	Gene Symbol	Gene Name	Accession Number
7.8	Edg8	Mus musculus endothelial differentiation, sphingolipid G-protein-coupled receptor, 8 (Edg8)	NM_053190.1
6.5	Cx3cr1	Mus musculus chemokine (C-X3-C) receptor 1 (Cx3cr1)	NM_009987.2
6.2	Gzma	Mus musculus granzyme A (Gzma)	NM 010370.1
4.3	Kirg1	Mus musculus killer cell lectin-like receptor subfamily G, member 1 (Klrg1)	NM 016970.1
3.8	Zfhx1b	Mus musculus zinc finger homeobox 1b (Zfhx1b)	NM 015753.2
3.7	Galnt10	Mus musculus UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 10 (Ga	NM 134189.2
3.7	Irak3	Mus musculus interleukin-1 receptor-associated kinase 3 (Irak3)	NM 028679.2
3.5	Sema4a	Mus musculus sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplas	NM_013658.2
3.5	Cxcr6	Mus musculus chemokine (C-X-C motif) receptor 6 (Cxcr6)	NM_030712.1
3.1	Esm1	Mus musculus endothelial cell-specific molecule 1 (Esm1)	NM_023612.3
3.0	C630004H02Rik	Mus musculus RIKEN cDNA C630004H02 gene (C630004H02Rik)	NM_175454.1
3.0	2310016C16Rik	Mus musculus RIKEN cDNA 2310016C16 gene (2310016C16Rik)	NM_027127.1
2.9	Ly6f	Mus musculus lymphocyte antigen 6 complex, locus F (Ly6f)	NM_008530
2.8	C130099A20Rik		NM_153420.1
2.8	Tnfsf6	Mus musculus tumor necrosis factor (ligand) superfamily, member 6 (Tnfsf6)	NM_010177.2
2.8	Prf1	Mus musculus perforin 1 (pore forming protein) (Prf1)	NM_011073.2
2.7	Dtx1	Mus musculus deltex 1 homolog (Drosophila) (Dtx1)	NM_008052.1
2.6	Bclp2	Mus musculus chitinase like protein 2 (Bclp2)	NM_170594.1
2.5	Anxa1	Mus musculus annexin A1 (Anxa1)	NM_010730.1
2.5	4632419K20Rik	Mus musculus RIKEN cDNA 4632419K20 gene (4632419K20Rik)	NM 199009.1
2.5	Cish	Mus musculus cytokine inducible SH2-containing protein (Cish)	NM_009895.2
2.5	Ccnd3	Mus musculus cyclin D3 (Ccnd3)	NM_007632.1
2.4	Prdm1	Mus musculus PR domain containing 1, with ZNF domain (Prdm1)	NM 007548.1
2.4	1700020L24Rik	Mus musculus RIKEN cDNA 1700020L24 gene (1700020L24Rik)	NM_025492.1
2.4	Arl7		NM_177305.2
2.4	II18rap	Mus musculus interleukin 18 receptor accessory protein (II18rap)	NM 010553.1
2.4	F2r	Mus musculus coagulation factor II (thrombin) receptor (F2r)	NM_010169.2
2.3	Pdgfb	Mus musculus platelet derived growth factor, B polypeptide (Pdgfb)	NM_011057.2
2.3	Ltb4r1	Mus musculus leukotriene B4 receptor 1 (Ltb4r1)	NM_008519.1
2.3	1110036O03Rik	Mus musculus RIKEN cDNA 1110036003 gene (1110036003Rik)	NM_176830.2
2.3	Bclp2	Mus musculus chitinase like protein 2 (Bclp2)	NM_170594.1
2.3	Hsd11b1	Mus musculus hydroxysteroid 11-beta dehydrogenase 1 (Hsd11b1)	NM_008288
2.2	Socs2	Mus musculus suppressor of cytokine signaling 2 (Socs2)	NM_007706.1
2.2	ldb2	Mus musculus inhibitor of DNA binding 2 (Idb2)	NM_010496.2
2.2	Tbx21	Mus musculus T-box 21 (Tbx21)	NM_019507.1
2.2	Sepn1	Mus musculus selenoprotein N, 1 (Sepn1)	NM_029100.1
2.2	Havcr2	Mus musculus hepatitis A virus cellular receptor 2 (Havcr2)	NM_134250.1
2.2	Actn2	Mus musculus actinin alpha 2 (Actn2)	NM_033268.2
2.1	Al646023	Mus musculus expressed sequence Al646023 (Al646023)	NM_198860.1
2.1	2310016C16Rik	Mus musculus RIKEN cDNA 2310016C16 gene (2310016C16Rik)	NM_027127.1
2.0	Stard10	Mus musculus START domain containing 10 (Stard10)	NM 019990.1

Data are averages from 4 independent samples of FACS sorted LCMV-specific WT and Blimp-1 CKO effector CD8 T cells from day 8 p.i. analyzed using Illumina Sentrix MouseRef-8 Expression BeadChips. Fold changes are the ratio of expression in CKO/WT cells (left table) or WT/CKO cells (right table). All fold changes are statistically significant, p < 0.05.

SUPPLEMENTARY METHODS

Blimp-1:YFP BAC transgenic mouse

For the construction of the Blimp-1:YFP BAC transgenic mouse, a bacterial artificial chromosome (BAC) containing Blimp-1 gene was used to generate a Blimp-1-YFP transgene. YFP cDNA was inserted in the second exon of Blimp-1 gene by homologous recombination and used to produce Blimp-1-YFP transgenic mice (Misulovin et al., 2001). Blimp-1-YFP transgenic mice were then backcrossed with C57Bl/6 mice for 15 generations.

Blimp-1 CKO mixed bone marrow chimeras

To generate mixed bone marrow chimeras, bone marrow from Ly5.1+ CD8 α -/- animals (Jackson Laboratories, Bar Harbor, ME) was mixed in a 90:10 ratio with bone marrow from either Ly5.2+ GzB-cre⁻; $Prdm1^{flox/flox}$ (WT) or Ly5.2+ GzB-cre⁺; $Prdm1^{flox/flox}$ (BMC CKO) and used to reconstitute lethally irradiated Ly5.1+ recipient mice. Two months later, these mice were infected with LCMV, and lymphocytes were isolated from tissues as described previously (Joshi et al., 2007).

CFSE labeling

For CFSE experiments, CD8 T cells were purified by magnetic bead depletion (Polyscienes Inc, Warrington, PA) of non-CD8 T cells using antibodies to CD4 (GK1.5), B220 (TIB164), and H-2^b (TIB120). Purified CD8 T cells (~80-90% pure) were then labeled with CFSE, as previously described (Joshi et al., 2007), and either cultured for 2 days with naïve congenic B6 feeder cells plus LCMV GP₃₃₋₄₁ peptide or ~1e6 LCMV-specific cells were transferred into Rag1-/- or γ-irradiated (750 RAD) recipient mice.

Surface and intracellular staining and antibodies.

All antibodies were purchased from e-biosciences (San Diego, CA) except anti-Granzyme B PE (Caltag, Burlingame, CA), anti-Bcl-2 FITC (BD, San Jose, CA), and anti-KLRG1 (2F1). Class I MHC tetramers were generated as described previously (Kaech et al., 2003). All flow cytometry was analyzed on a FACSCalibur or LSRII (BD) with FloJo software (Treestar, San Carlos, CA).

Microarray Hybridization and Analysis

0.1 mg of total RNA from each sample was hybridized to Illumina Sentrix MouseRef-8 Expression BeadChips (Illumina, San Diego, CA) as described previously (Magnusdottir et al., 2007). The resulting data were imported into the Spotfire Decision Site functional genomics software suite and filtered from background signal values at P > 0.95 using statistical models employed in Illumina BeadStudio software. Differentially expressed genes were further identified in the resulting dataset using SAM hypothesis testing statistics followed by Storey's false-discovery rate correction (Tusher et al., 2001). In this analysis, FDR cutoff less then 0.086 produced 350 differentially expressed genes (with 15 genes being possible false calls at 90% percentile). Genes with the expression changes smaller than 2-fold were further eliminated, and remaining genes are presented in Table 1 and Table S1. These data are currently in the process of being uploaded to NCBI Gene Expression Omnibus and a URL will be forthcoming. Predicted Blimp-1 binding sites were assessed using Genomatix software (www.genomatix.de).

qRT-PCR Methods

For qRT-PCR, RNA was isolated from ~2 x 10⁵ FACS sorted cells, cDNA was synthesized using SSRTII (Invitrogen, Carlsbad, CA) or MMuLV (New England Biolabs, Ipswitch, MA), real time PCR was performed and relative fold differences were calculated as described previously (Joshi et al., 2007). All expression values were

normalized to levels of the ribosomal protein, L9. Primers used are as follows: *L9*: 5'-TGAAGAAATCTGTGGGTCG-3' (forward), 5'-GCACTACGGACATAGGAACT-3' (reverse); *Prdm1*: 5'-GACGGGGGTACTTCTGTTCA-3' (forward), 5'-GGCATTCTTGGGAACTGTGT-3' (reverse); *Bcl6*: 5'-CTGCAGATGGAGCATGTTGT-3' (forward), 5'-cacccgggagtatttctcag-3' (reverse); *Tcf7*: 5'-CGCTGCATAACAAGCC-3' (forward), 5'-CCAGCTCACAGTATGGG-3'; *Tbx21*: 5'-CAACAACCCCTTTGCCAAAG-3' (forward), 5'-TCCCCCAAGCAGTTGACAGT-3'.

SUPPLEMENTARY REFERENCES

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